



Yang-Chang WU  
Application No. 10/005,921

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DEC 30 2002  
USPTO CENTER 100/2900

**IN THE TITLE:**

*Changed  
in PALM*

Please amend the title as follows:

-- CYTOTOXIC ANNONACEOUS ACETOGENINS FROM *ANNONA MURICATA* --

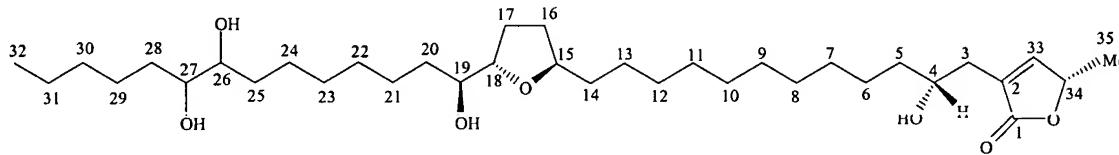
**IN THE CLAIMS:**

*A.E.*

Please amend claim 1 as follows:

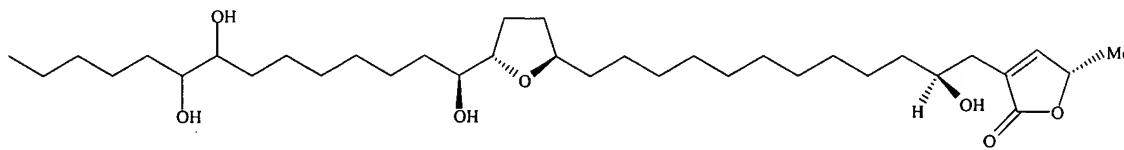
1. (Amended) Isolated and purified Annonaceous acetogenin compounds having the structures of a-g, wherein

a. muricin A has the formula of:



*B5*  
said muricin A having an  $\alpha, \beta$ -unsaturated  $\gamma$ -lactone with a hydroxyl group at C-4, a mono-THF ring placed between C-15 and C-18 with one flanking hydroxyl in a threo conformation, two methylene groups of the mono-THF ring corresponding to a trans conformation, two hydroxyl groups at C-26 and C-27 as vicinal diol assigned as threo based, and the stereochemistry at C-34 on the  $\gamma$ -lactone fragment performed in (S)-configuration;

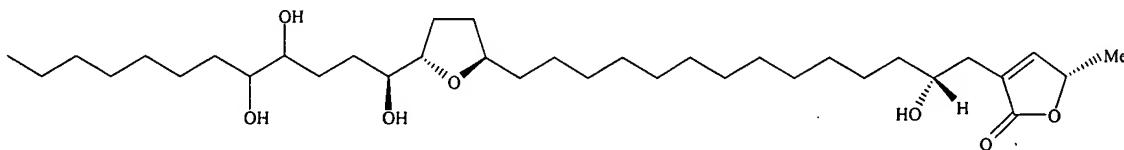
b. muricin B has the formula of:



said muricin B having an  $\alpha, \beta$ -unsaturated  $\gamma$ -lactone with a hydroxyl group at C-4, a mono-THF ring placed between C-15 and C-18 with one flanking hydroxyl in a trans/threo conformation, two methylene groups of the mono-THF ring corresponding to a trans

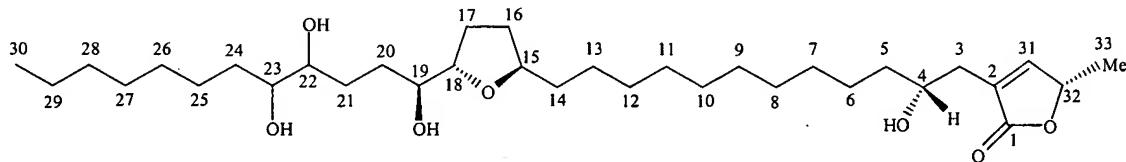
conformation, two hydroxyl groups at C-26 and C-27 as vicinal diol assigned as threo based, and the stereochemistry at C-34 on the  $\gamma$ -lactone fragment performed in (S)-configuration;

c. muricin C has the formula of:



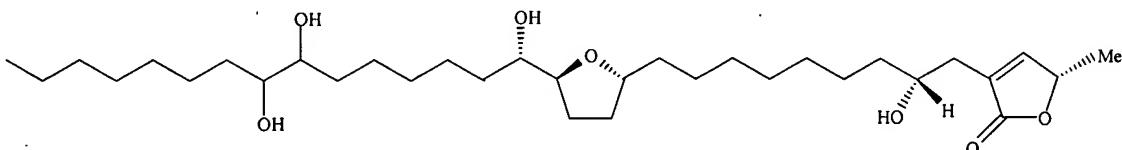
said muricin C having an  $\alpha, \beta$ -unsaturated  $\gamma$ -lactone with a hydroxyl group at C-4, a mono-THF ring placed between C-17 and C-20 with one flanking hydroxyl in a trans/threo or threo/trans conformation, two hydroxyl groups at C-24 and C-25 as vicinal diol assigned as threo based, and the stereochemistry at C-34 on the  $\gamma$ -lactone fragment performed in (S)-configuration;

d. muricin D has the formula of:



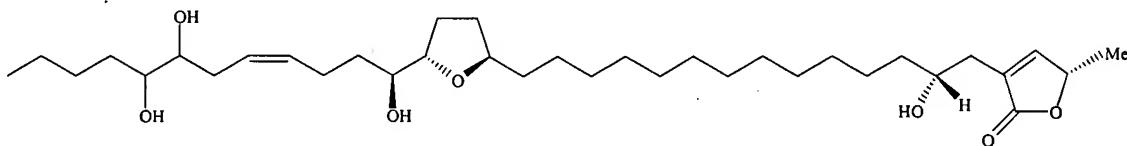
said muricin D having an  $\alpha, \beta$ -unsaturated  $\gamma$ -lactone with a hydroxyl group at C-4 position, a mono-THF ring placed between C-15 and C-18 with one flanking hydroxyl in a threo/trans conformation, two hydroxyl groups at C-22 and C-23 as vicinal diol assigned as threo based.;

e. muricin E has the formula of:



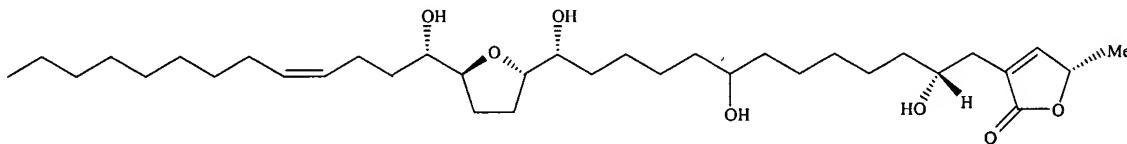
said muricin E having an  $\alpha, \beta$ -unsaturated  $\gamma$ -lactone with a hydroxyl group at C-4 position, a mono-THF ring placed between C-12 and C-15 with one flanking hydroxyl in a threo/trans conformation, two hydroxyl groups at C-22 and C-23 as vicinal diol assigned as threo based;.

f. muricin F has the formula of:



said muricin F having an  $\alpha, \beta$ -unsaturated  $\gamma$ -lactone with a hydroxyl group at C-4, a mono-THF ring placed between C-17 and C-20 with one flanking hydroxyl in a threo/trans conformation, two hydroxyl groups at C-22 and C-23 as vicinal diol assigned as threo based, and a double bond determined at C-24/C-25; and

g. muricin G has the formula of:



said muricin G having an  $\alpha, \beta$ -unsaturated  $\gamma$ -lactone with a hydroxyl group at C-4, a mono-THF ring placed between C-16 and C-19 with one flanking hydroxyl in a threo/trans/threo conformation, one hydroxyl groups formed at C-10, a double bond determined at C-23/C-24, and the stereochemistry at C-34 on the  $\gamma$ -lactone fragment performed in (S)-configuration.

Please amend claim 2 as follows:

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2. (Amended) A method for isolating and purifying the Annonaceous acetogenins compounds according to claim 1 comprising:

extracting muricins from *Annona muricata* seeds with MeOH to obtain a MeOH extract at room temperature;

evaporating and partitioning the MeOH extract in a CHC<sub>13</sub> and aqueous mixture, whereby said Annonaceous acetogenins compounds are in the CHC<sub>13</sub> layer of the CHC<sub>13</sub> and aqueous mixture; and

further separating the Annonaceous acetogenins compounds of said CHC<sub>13</sub> layer by column chromatography.

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B<sup>1</sup>  
Please cancel claims 3-4.

B<sup>2</sup>  
Please amend claim 5 as follows:

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5. (Amended) An anti-tumor composition comprising an effective amount of at least one of the Annonaceous acetogenins compounds according to claim 1.

B<sup>2</sup>

(Please amend claim 6 as follows:)

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6. (Amended) The Annonaceous acetogenins compounds as claimed in claim 1, wherein the Annonaceous acetogenins compounds are used for treatment of patients having a tumor.

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B<sup>2</sup>  
Please amend claim 9 as follows:

*B3*  
9. (Amended) A method for treating hepatoma cancer comprising administering to a patient afflicted with hepatoma cancer an effective amount of a pharmaceutical composition comprising at least one Annonaceous acetogenins compounds according to claim 1 and a pharmaceutically acceptable salt and ester in combination with pharmaceutically acceptable carrier, auxiliary or excipient.

Please add new claims 10-18 as follows:

**New Claim 10.** The isolated and purified Annonaceous acetogenins compounds according to claim 1, wherein said compound is isolated from *Annona muricata*.

**New Claim 11.** The isolated and purified Annonaceous acetogenins compounds according to claim 10, wherein said compound is isolated from seeds of *Annona muricata*.

**New Claim 12.** The method according to claim 2, wherein said column chromatography is an Si gel column.  
*B4*

**New Claim 13.** The method according to claim 12, wherein said Annonaceous acetogenins compounds are eluted from the Si gel column by a gradient comprising *n*-hexane-CHCl<sub>3</sub> and CHCl<sub>3</sub>-MeOH.

**New Claim 14.** The method according to claim 13, wherein said Annonaceous acetogenins compounds are further purified by a reversed-phase high performance liquid chromatography.

**New Claim 15.** The method according to claim 13, wherein said CHCl<sub>3</sub> layer is separated into ten fractions by the Si gel column.